Kidney International, Vol. 31 (1987), pp. 1121–1125

Effects of amiloride in the medullary collecting duct of rat kidney

HARALD SONNENBERG, URSULA HONRATH, and DOUGLAS R. WILSON

Department of Physiology, University of Toronto, Toronto, Ontario, Canada

Effects of amiloride in the medullary collecting duct of rat kidney. The in vivo microcatheterization technique was used to study amiloride–induced transport alterations in the inner medullary collecting duct. Amiloride treated rats (0.1 mg/hr) had significant diuresis and natriuresis, as well as antikaliuresis, compared to untreated controls. The relative decrease in potassium excretion was associated with a significant rise in plasma potassium concentration. Net sodium transport in the duct was decreased from 83 + 3 to 46 + 6 per cent of delivered load, as a result of amiloride treatment. Smaller, but statistically significant, reductions ($P < 0.01$) were seen for fluid and chloride reabsorptions (from 66 + 3 to 51 + 4%, and from 72 + 4 to 52 + 5%, respectively). Potassium reabsorption increased from 15 + 8 to 61 + 6% of delivered load. The data indicated that amiloride natriuresis is determined primarily by inhibition of sodium reabsorption in the medullary collecting duct, probably due to blockade of a specific Na channel. The antikaliuresis, on the other hand, appears to be due to inhibition of secretion both in upstream nephron segments and in the duct itself.

The pyrazine derivative diuretic, amiloride, causes modest diuresis and natiuresis, coupled with marked antikaliuresis [1]. In micropuncture experiments in the rat, amiloride completely suppressed potassium (K) secretion at the distal tabular level with little detectable alteration of sodium (Na) reabsorption [2]. These results suggest that the major natriuretic effect is localized downstream to the distal tubule. Isolated cortical collecting tubules from rabbit kidney showed complete inhibition of Na and K transport with addition of the drug to the lumen [3]. In both nephron segments amiloride reduced lumenal electronegativity [2, 3], apparently by blocking an electrogenic sodium channel in the apical membrane [4]. The mechanism of amiloride–induced natriuresis and antikaliuresis thus involves inhibition of electrogenic Na transport in late distal and cortical collecting tubules, with consequent reduction of K secretion.

It is not clear whether transport changes in the medullary collecting system contribute to the renal response to amiloride. Since, in contrast with the upstream segments, there is no significant electrical potential difference across medually duct epithelium [5], amiloride might be expected to have no effects on sodium and potassium transport at this site. However, using the in vivo shrinking droplet technique, a major inhibition of reabsorptive volume flux was found in rat papillary collecting duct [6]. We decided, therefore, to use the in vivo microcatheterization technique to assess water, sodium, chloride, and potassium transport along the inner medullary collecting duct in the presence and absence of amiloride.

Received for publication August 11, 1986
and in revised form December 1, 1986

Methods

Male Sprague Dawley rats (weight range, 223 to 354 g) were used for clearance and microcatheterization experiments as previously described [7–9]. The animals were anesthetized with Inactin (10 mg/100 g body wt, i.p.), and body temperature was kept near 38°C. After tracheostomy, jugular vein and femoral artery cannulation allowed intravenous infusion and blood pressure monitoring and sampling. The left kidney was prepared for microcatherization and urine collection. On completion of surgery 1.25 ml of a mixture of 0.15 M Nacl and fresh homologous rat plasma (3:1) was given as a priming dose, followed by constant intravenous infusion of the same solution at 1.25 ml/hr. The solution contained 25 μCi/ml of ^{3}H-inulin. Two groups of rats were studied. The first, control series (N = 17), received the intravenous infusion as described. The second, experimental series (N = 17) was treated identically, except that amiloride (0.08 mg/ml) was added to the infusion solution, resulting in an estimated plasma concentration of the drug in the range of 10^{-6} M.

In both groups, a one hour equilibration period was followed by four consecutive 30 minute urine collection periods. Arterial blood samples (0.1 ml) were taken in the middle of each collection. During this two hour collection period microcatheterization was used to obtain samples of tubular fluid from medullary collecting ducts [7–9]. In general, three separate duct systems were each sampled near the papillary tip (end of duct) and near the border between inner and outer stripes of outer medulla (beginning). The average depths of insertion in the two groups were as follows: control—beginning = 0.55 + 0.03 mm, end = 5.91 + 0.55 mm; experimental—beginning = 0.64 + 0.03 mm, end = 6.13 + 0.55 mm. Inner medullary lengths in these series were 4.77 + 0.16 and 5.00 + 0.09 mm, respectively. It was thus possible to assess transport along the whole extent of the inner medullary collecting system.

Concentrations of ^{3}H-inulin, sodium, chloride, and potassium in plasma, urine, and tubular fluid were measured as described previously, and used to calculate fractional and absolute deliveries of water and electrolytes to the beginning and end of the medullary duct in individual rats [7–9]. Net transport along the duct was determined as the difference between beginning and

Table 1. Renal function in control and amiloride treated rats

	V μl/min/g	$U_{Na}V$ nmol/min/g	$U_{Cl}V$ nmol/min/g	U_KV nmol/min/g	GFR mL/min/g
Control	9.1	520	805	772	1.01
(N = 17)	±0.94	±88	±130	±97	±0.04
Amiloride	14.0[a]	2178[a]	1756[a]	121[a]	0.91
(N = 17)	±1.1	±177	±181	±31	±0.06

[a] Significant difference ($P < 0.01$) between series (unpaired t-test)

end delivery. Statistical assessment of the data included linear regression analysis, analysis of co-variance, as well as paired and unpaired t-tests, where indicated. Since renal function did not change significantly over the two hour collection period in either group, averages ± SEM of overall renal as well as tubular fluid data were calculated for each animal and used for statistical comparisons.

Results

Plasma sodium and chloride concentrations were not different between control and amiloride treated groups (P_{Na} = 143 + 1 vs. 142 + 1 mM, P_{Cl} = 107 + 1 vs. 107 + 1 mM). Average plasma potassium concentrations, however, were higher in the latter (5.5 + .1 vs. 4.5 + .1 mM, $P < 0.01$), apparently due to the marked reduction of kaliuresis with drug treatment (Table 1). In addition, as shown in Table 1, diuresis increased modestly but significantly, natriuresis rose fourfold, and chloriuresis doubled following amiloride treatment. Glomerular filtration rate did not change significantly.

Tubular deliveries of fluid and electrolytes to the beginning and end of the inner medullary collecting duct were averaged for individual animals of control and amiloride groups, and are shown in Table 2. Comparison of the calculated tubular loads near the end of the duct with actual excretion (Table 1) shows good correspondence, demonstrating that the data obtained from the catheterized ducts are representative of overall kidney function. There were no statistically significant differences in absolute fluid and sodium chloride deliveries to the beginning of the duct in the two groups, indicating that amiloride had no major upstream diuretic or saluretic effects. However, as expected, potassium delivery was markedly reduced by the drug. Although there was significant fluid and electrolyte reabsorption along the inner medullary duct in both series, amiloride significantly reduced the level of absolute water and sodium transport at this site, compared to control. These effects of the drug become more obvious when fractional reabsorptions of loads delivered to the collecting duct are calculated. Fractional sodium transport was reduced from 83 + 3 to 46 + 6% ($P < 0.01$, unpaired t), while the inhibition of water and chloride transport, although not as striking, was highly significant statistically (66 + 3 to 51 + 4 and 72 + 4 to 52 + 5%, respectively; $P < 0.01$).

The action of amiloride on potassium transport along the medullary collecting duct is not as clear cut. Absolute K reabsorption appeared greater in control than drug–treated animals (Table 2), although this difference was not statistically significant. However, consequent to the reduction of K delivery in the experimental series, fractional potassium reabsorption was markedly increased from 15 + 8 to 61 + 6% ($P < 0.01$).

Since normally there is a direct correspondence between the deliveries of fluid and sodium to the collecting duct and absolute downstream reabsorption [10, 11], the effect of amiloride on such load reabsorption relationships is shown in Figures 1 to 4. Analysis of co-variance was used to assess statistical significance of differences in slopes and intercepts between the two groups. As can be seen in Figure 1, there was a linear relationship between sodium delivery and reabsorption among the individual animals of the control series. In the experimental series, a significant downward displacement ($P < 0.01$) of the calculated regression line demonstrates the marked amiloride induced inhibition of sodium reabsorption at any given delivery. The displacements of the chloride (Fig. 2) and fluid reabsorption curves (Fig. 3) were not as large as for sodium, although still statistically significant ($P < 0.05$). For potassium, reabsorption increased in delivered load in control rats (Fig. 4), although at low deliveries the regression line indicated net secretion of the ion into duct fluid. This secretory component was eliminated by drug treatment, as shown by the upward displacement of the experimental regression line ($P < 0.01$).

Discussion

As expected, amiloride caused natriuresis and antikaliuresis in the present experiments. Inhibition of sodium reabsorption in the cortical collecting tubule has been shown previously [3]. However, such inhibition is insufficient to fully explain the natriuretic response in the present experiments, since the (statistically not significant) increase in salt delivery to the inner medullary collecting system could not account for the observed increase in sodium chloride excretion (Tables 1 and 2). The demonstrated reduction of transport in this terminal nephron segment, therefore, contributes in a major way to amiloride natriuresis. The explanation for the insignificant upstream effect can probably be found in the small absolute magnitude of Na transport in the cortical collecting tubule under normal conditions [3]. By contrast, the reduction of potassium delivery to the duct is proportional to the reduction of K excretion, consistent with inhibition of secretion of this ion in distal and cortical collecting tubules [2, 3]. These segments, therefore, contribute in a major way to amiloride induced antikaliuresis. In addition, however, the diuretic also eliminates a K-secretory component of transport in the inner medullary duct (Fig. 4), thus potentiating the potassium sparing action of the drug.

The mechanism of the amiloride induced inhibition of collecting duct sodium transport is not established by the present experiments. If amiloride blocks an electrogenic Na channel in this epithelium as it does in upstream nephron segments, normal sodium movement through this channel should be linked to corresponding Cl reabsorption, since no transtubular electrical gradient is generated [5]. Alternately, amiloride could affect a sodium–hydrogen antiporter, as it does in proximal tubule [12, 13], thick ascending limb [14], and cortical collecting tubule [15]. Although the plasma level of amiloride in our animals is less than that required for the effect on the antiporter, concentration of drug in the collecting duct lumen by upstream fluid reabsorption could bring it into the effective range (10^{-3} to 10^{-4} M) [12–15]. However, using the shinking droplet technique combined with peritubular perfusion, Ullrich and Papavassiliou [6] found that 10^{-4} M amiloride in the lumen resulted in similar quantitative inhibition of sodium reabsorption (to 40% of con-

Table 2. Absolute loads near beginning (B) and end (E) of medullary collecting duct in control and amiloride treated rats

	V *μl/min/g*			Na *nmol/min/g*			Cl *nmol/min/g*			K *nmol/min/g*		
	B	E	Δ	B	E	Δ	B	E	Δ	B	E	Δ
Control	37.1	12.2	24.9[d]	3393	597	2796[d]	4493	1260	3232[d]	1183	864	320[c]
(N = 17)	±3.9	±1.6	±3.0	±495	±156	±390	±700	±297	±553	±176	±114	±135
Amiloride	33.4	16	17.4[a,d]	3903	1993[b]	1891[b,d]	3904	1778	2126[d]	294[b]	130[b]	163[d]
(N = 17)	±2.2	±1.6	±2.0	±370	±223	±322	±371	±220	±325	±52	±42	±29

[a,b] Significant difference ($P < 0.05, 0.01$) between series (unpaired t)
[c,d] Significant difference ($P < 0.05, 0.01$) between B and E within series (paired t)

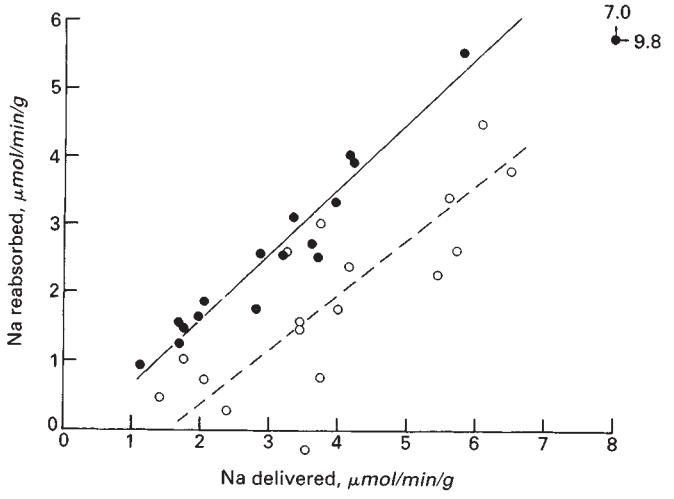

Fig. 1. *Absolute amounts of sodium reabsorbed along the inner medullary collecting duct of individual control (○) and amiloride treated rats (●), in relation to sodium deliveries to the duct.* Statistical regression lines for the control (solid line; correlation coefficient, r = 0.96, $P < 0.01$) and experimental series (broken line; correlation coefficient, r = 0.79, $P < 0.01$) are shown.

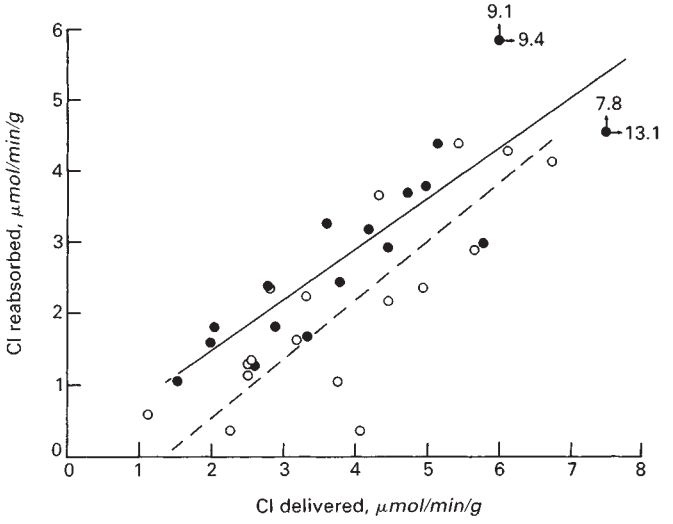

Fig. 2. *Chloride delivery and reabsorption in the medullary collecting duct.* Symbols and explanations as for Figure 1. Regression statistics are given as follows: control, r = 0.92, $P < 0.01$; experimental, r = 0.81, $P < 0.01$.

trol) to that observed in the present experiments following amiloride (to 55% of control). They ascribed this effect to blockage of an amiloride sensitive channel, rather than hydrogen ion exchange, since removal of bicarbonate from lumenal fluid only reduced volume flux to 77% of control. This interpretation of the natriuretic mechanism of the drug is supported by the following considerations: if the difference in duct sodium reabsorption between control and experimental groups (Fig. 1) is due to Na-H exchange, such exchange represents a significant fraction of total Na transport in this nephron segment. In normal rats, therefore, at any given delivery one would expect a relatively greater net reabsorption of measured cations (Na + K) than that of anion (Cl), assuming that transtubular movement of the latter will maintain electroneutrality. Furthermore, in amiloride treated rats the ratio of cation to chloride reabsorption should tend to unity. However, as shown in Figure 5A, in control animals the slope of the regression line relating Na + K reabsorption on the one hand, and Cl reabsorption on the other, was not different from unity, and did not change in amiloride treated rats (Fig. 5B). In agreement with results in proximal tubule [13] and ascending limb [14], these data confirm, therefore, that Na-H exchange is not a major component contributing to net sodium reabsorption in the medullary collecting duct

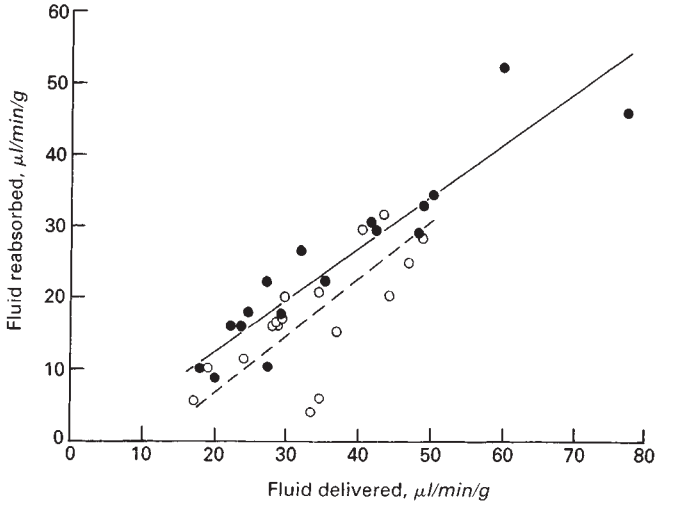

Fig. 3. *Fluid delivery and reabsorption in the medullary collecting duct.* Symbols and explanations as for Figure 1. Regression statistics are given as follows: control, r = 0.92, $P < 0.01$; experimental, r = 0.72, $P < 0.01$.

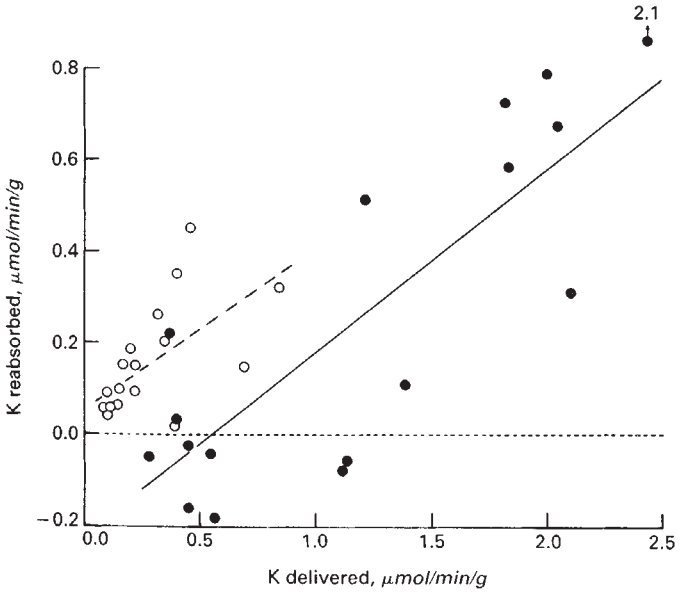

Fig. 4. *Potassium delivery and reabsorption in the medullary collecting duct.* Symbols and explanations as for Figure 1. Regression statistics are given as follows: control, $r = 0.76$, $P < 0.01$; experimental, $r = .59$, $P < 0.05$.

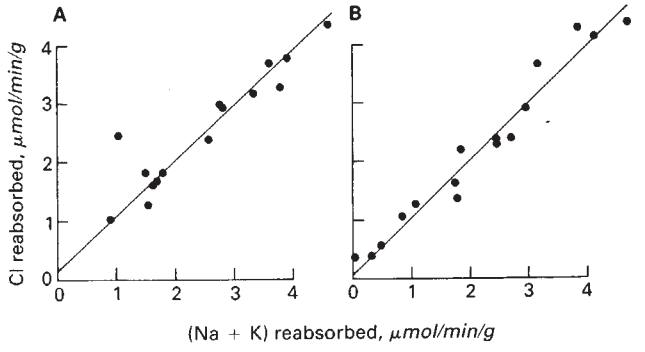

Fig. 5. *Absolute levels of (Na + K) vs. Cl reabsorption in the medullary collecting duct of individual rats.* **A.** Control series. **B.** amiloride series. The solid lines indicate the statistical regression lines in the respective groups. A. Slope, b = 0.97; correlation coefficient, $r = 0.97$ ($P < 0.01$) B. b = 0.98, $r = 0.98$ ($P < 0.01$).

[16]. In addition, they suggest that amiloride natriuresis is due to blockage of a sodium channel in the duct epithelium, rather than to inhibition of sodium–hydrogen countertransport.

The present potassium data demonstrate for the first time that there is load-dependent K reabsorption in the medullary collecting duct, as there is for sodium [10, 11]. The superimposition of a secretory component (Fig. 4) explains the lack of net transport one usually finds in antidiuretic conditions [7–9]. Amiloride blocks this secretory component, thus potentiating the antikaliuretic effect. The mechanism of the amiloride effect on potassium remains unresolved. However, the data do explain the relatively greater inhibition of sodium, compared to chloride and fluid transport, since enhanced reabsorption of chloride (as KCl) could compensate in part for the inhibition of the drug-induced NaCl reabsorption.

The present study demonstrates that the inner medullary collecting duct has amiloride-sensitive transport sites. In addition, the greater inhibitory effect of chlorothiazide on chloride, compared to sodium transport at this site [17] suggests an effect on chloride conductance analogous to that proposed for Amphiuma distal tubule [18]. Finally, furosemide inhibits sodium chloride reabsorption along the duct [10,19], suggesting the presence of a luminal carrier analogous to that in the thick ascending limb. These observations suggest multiple mechanisms of salt reabsorption in the medullary collecting system. It has not yet been tested whether amiloride and chlorothiazide would have additive effects. However, furosemide alone can completely block net NaCl transport at this site [10, 19], an effect not consonant with multiple carriers. One might speculate that the furosemide effect is mediated by non-carrier-dependent mechanisms, possibly via peritubular hemodynamic alterations. Obviously, the complex interactions determining salt reabsorption in the terminal nephron segment in vivo will require further study.

In summary, we have shown that amiloride natriuresis is determined largely by inhibition of sodium reabsorption in the medullary duct, whereas the antikaliuresis is primarily dependent on upstream inhibition of K secretion. In addition amiloride eliminates a K secretory component in the duct itself.

Acknowledgments

This study was supported, in part, by grants MT-4043 (HS) and MT-2836 (DRW) from the Medical Research Council of Canada.

Reprint requests to Dr. Harald Sonnenberg, Department of Physiology, Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A8.

References

1. Baba WI, Lant AF, Smith AJ, Townsend MM, Wilson GW: Pharmacological effect in animals and normal human subjects of the diuretic amiloride hydrochloride (mk-870). *Clin Pharmacol Ther* 9:318–327, 1968
2. Duarte CG, Chomety F, Giebisch G: Effect of amiloride, ouabain, and furosemide on distal tubular function in the rat. *Am J Physiol* 221:632–639, 1971
3. Stoner LC, Burg MB, Orloff J: Ion transport in cortical collecting tubule; effect of amiloride. *Am J Physiol* 227:453–459, 1974
4. O'Neil RG, Boulpaep EL: Effect of amiloride on the apical cell membrane cation channels of a sodium-absorbing, potassium-secreting renal epithelium. *J Membr Biol* 50:365–387, 1979
5. Hayslett JP, Backman KA, Schon DA: Electrical properties of the medullary collecting duct in the rat. *Am J Physiol* 230:F258–F264, 1980
6. Ullrich KJ, Papavassiliou F: Sodium reabsorption in the papillary collecting duct of rats. Effect of adrenalectomy, low Na^+ diet, acetazolamide, HCO_3^--free solutions and of amiloride. *Pflügers Arch* 379:49–52, 1979
7. Sonnenberg H: Medullary collecting duct function in antidiuretic and in salt or water diuretic rats. *Am J Physiol* 226:501–506, 1974
8. Sonnenberg H, Wilson DR: The role of the medullary collecting duct in postobstructive diuresis. *J Clin Invest* 57:1564–1574, 1976
9. Wilson DR, Sonnenberg H: Medullary collecting duct function in the remnant kidney before and after volume expansion. *Kidney Int* 15:487–501, 1979
10. Sonnenberg H: Effects of furosemide, acetazolamide, and mannitol on medullary collecting duct function in the rat kidney. *Pflügers Arch* 373:113–123, 1978
11. Wilson DR, Honrath U, Sonnenberg H: Effect of acetylcholine

and secretin on medullary collecting duct function in the rat. *Can J Physiol Pharmacol* 64:62–65, 1986
12. KINSELLA JL, ARONSON PS: Properties of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am J Physiol* 238: F461–F469, 1980
13. HOWLIN KJ, ALPERN RJ, RECTOR FC JR: Amiloride inhibition of proximal tubular acidification. *Am J Physiol* 248:F773–F778, 1985
14. GOOD DW: Sodium–dependent bicarbonate absorption by cortical thick ascending limb of rat kidney. *Am J Physiol* 248:F821–F829, 1985
15. MCKINNEY TH, BURG MB: Bicarbonate absorption by rabbit cortical collecting tubules in vitro. *Am J Physiol* 242:F141–F145, 1978
16. BENGELE HH, MCNAMARA ER, ALEXANDER EA: Effect of acute thyroparathyroidectomy on nephron acidification. *Am J Physiol* 246:F569–F574, 1984
17. WILSON DR, HONRATH U, SONNENBERG H: Thiazide diuretic effect on medullary collecting duct function in the rat. *Kidney Int* 23:711–716, 1983
18. HANSEN L, SCHILLING AR, WIEDERHOLT M: Effect of calcium, furosemide, and chlorothiazide on net volume reabsorption and basolateral membrane potential of the distal tubule. *Pflügers Arch* 389:121–126, 1981
19. WILSON DR, HONRATH U, SONNENBERG H: Furosemide action on collecting ducts: Effect of prostaglandin synthesis inhibition. *Am J Physiol* 244:F666–F673, 1983